

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Thomas H. TURPEN

Application Serial No. Not Yet Assigned

Filed: August 14, 2001

For: **VIRAL AMPLIFICATION OF  
RECOMBINANT MESSENGER  
RNA IN TRANSGENIC PLANTS**

Group Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

Attorney's Docket No:  
00801.0103.DVUS02**PRELIMINARY AMENDMENT**Commissioner for Patents  
Washington, D.C. 20231

Sir:

**AMENDMENT****In the Specification:**

On page 1, after line 5, insert:

**--CROSS-REFERENCE TO RELATED APPLICATIONS**

The present application is a divisional of application Serial No. 09/414,916, filed January 31, 2001, which is a continuation of Application Serial No. 08/336,724 filed November 9, 1994, now U.S. Patent No. 5,965,794, which is a continuation of application Serial No. 07/997,733, filed December 30, 1992, now abandoned.--

On page 11, line 12, after "replicon" insert -- (SEQ ID No: 1) --.

On page 18, line 4, after "gene" insert -- (encoding the 30-kDA movement protein, the amino acid sequence of which is depicted by SEQ ID NO: 2) --.

On page 18, line 29, after "Figure 6" insert -- (SEQ ID NO: 1) --.

One page 18, line 29, delete “(CAT)” and insert --, encoding CAT (the amino acid sequence of which is depicted by SEQ ID NO: 3), --.

**In the Claims**

Please cancel Claims 1-11, and 14-25.

Please amend the following claims:

12. (Amended) A heterologous protein expressed using [the] a replicon [of Claim 1] derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants.
13. (Amended) A heterologous protein expressed using [the] a replicon [of Claim 7] derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein said replicon codes for a viral movement protein, wherein said replicon is capable of moving the replicon-encoded genes away from the site of infection and is capable of systemic expression.

**REMARKS**

**The Amendments**

The new paragraph inserted in page 1 is to provide priority information.

The amendments on pages 11 and 18 are to insert SEQ ID information.

Claims 1-11, and 14-25 are cancelled.

Claims 12 and 13 are amended. Support for Claims 12 and 13 is found, for example, in Claims 1, 5, 7, 12, and 13 as originally filed in the parent application.

No new matter is added in any of the above amendments and the Examiner is respectfully requested to enter the amendments.

Respectfully submitted,

Date: August 14, 2001



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

On page 1, after line 5, insert:

**--CROSS-REFERENCE TO RELATED APPLICATIONS**

The present application is a divisional of application Serial No. 09/414,916, filed January 31, 2001, which is a continuation of application Serial No. 08/336,724 filed November 9, 1994, now U.S. Patent No. 5,965,794, which is a continuation of application Serial No. 07/997,733, filed December 30, 1992, now abandoned. --

In the paragraph on Page 11, line 12:

Fig. 6 is the sequence of the RNA replicon (SEQ ID NO: 1) described in Example 1.

In the paragraph on page 18, line 3:

In this construction, it is desired to place the 30-kDA movement protein gene (encoding the 30-kDA movement protein, the amino acid sequence of which is depicted by SEQ ID NO: 2) at precisely the same position as the replicase gene (relative to 5' replication origin in the wild type TMV genome, See Figure 5). To accomplish this, a NdeI site is introduced at the start codon of each gene by PCR-based mutagenesis using synthetic primers and unique adjacent cloning sites. A 270 bp mutagenesis product containing the internal NdeI site from the PCR primer is subcloned using the EcoRV site in the cauliflower mosaic virus 35S promoter and the HindIII site in the 30-kDa protein gene. The ligation product is then sequence verified.

In the paragraph on page 18, line 16:

The 3' segment of the replicon, containing the CAT gene will be placed adjacent to the 3'-ribozyme as a HindIII-NsiI fragment from the transient TMV vector pTMVS3CAT28 (Figure 5). In the final cloning step, the 5' portion of the transgene and the 3' portion will be subcloned into the unique BamHI site of the plant transformation vector pAP2034 (Velton and Schell, NAR 13:6981-6998 (1985) as a BglII-BamHI fragment described previously (Turpen, T.

H., Ph.D. Dissertation, University of California, Riverside, pp. 88-132 (1992)). The sequence of the replicon RNA, produced by host transcription, RNA processing, and replication in the presence of a helper virus, is given in Figure 6 (SEQ ID NO: 1). Thus, the foreign gene [(CAT)] encoding CAT (the amino acid sequence of which is depicted by SEQ ID NO: 3), is placed on a RNA viral replicon, under control of the coat protein subgenomic promoter for messenger RNA synthesis (located at a 3' end of the movement protein gene).

### **In the Claims**

Please cancel Claims 1-11 and 14-25.

Please amend the following claims:

12. (Amended) A heterologous protein expressed using [the] a replicon [of Claim 1] derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants.

13. (Amended) A heterologous protein expressed using [the] a replicon [of Claim 7] derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein said replicon codes for a viral movement protein, wherein said replicon is capable of moving the replicon-encoded genes away from the site of infection and is capable of systemic expression.

**CLEAN VERSION WITH CHANGES INCORPORATED**

**In the Specification:**

On page 1, the following new paragraph is inserted after line 5:

**CROSS-REFERENCE TO RELATED APPLICATIONS**

The present application is a divisional application of U.S. Application Serial No. 09/414,916, filed January 31, 2001, which is a continuation application of application Serial No. 08/336,724 filed November 9, 1994, now U.S. Patent No. 5,965,794, which is a continuation of application Serial No. 07/997,733, filed December 30, 1992, now abandoned.

On page 11, the paragraph starting on line 12:

Fig. 6 is the sequence of the RNA replicon (SEQ ID NO: 1) described in Example 1.

On page 18, the paragraph starting on line 3:

In this construction, it is desired to place the 30-kDA movement protein gene (encoding the 30-kDA movement protein, the amino acid sequence of which is depicted by SEQ ID NO: 2) at precisely the same position as the replicase gene (relative to 5' replication origin in the wild type TMV genome, See Figure 5). To accomplish this, a NdeI site is introduced at the start codon of each gene by PCR-based mutagenesis using synthetic primers and unique adjacent cloning sites. A 270 bp mutagenesis product containing the internal NdeI site from the PCR primer is subcloned using the EcoRV site in the cauliflower mosaic virus 35S promoter and the HindIII site in the 30-kDa protein gene. The ligation product is then sequence verified.

On page 18, the paragraph starting on line 16:

The 3' segment of the replicon, containing the CAT gene will be placed adjacent to the 3'-ribozyme as a HindIII-NsiI fragment from the transient TMV vector pTMVS3CAT28 (Figure 5). In the final cloning step, the 5' portion of the transgene and the 3' portion will be subcloned into the unique BamHI site of the plant transformation vector pAP2034 (Velton and Schell, NAR 13:6981-6998 (1985) as a BglII-BamHI fragment described previously (Turpen, T.

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### **In the Claims**

12. A heterologous protein expressed using a replicon derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants.

13. A heterologous protein expressed using a replicon derived from a chromosomally integrated transgene capable of expressing at least one foreign gene in plant cells, possessing replication origins with substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein the replicon is dependent for replication on a helper virus possessing trans-acting replication proteins, the replication proteins having substantial sequence homology to any plus sense, RNA virus capable of infecting plants, wherein said replicon codes for a viral movement protein, wherein said replicon is capable of moving the replicon-encoded genes away from the site of infection and is capable of systemic expression.